Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 9412–9416, December 1989
Genetics

Urate oxidase: Primary structure and evolutionary implications

(sequence comparison/copper binding/protein modification/nonsense mutations in humans)

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Communicated by James B. Wyngaarden, August 16, 1989

ABSTRACT **Urate oxidase, or uricase (EC 1.7.3.3), is a peroxisomal enzyme that catalyzes the oxidation of uric acid to allantoin in most mammals. In humans and certain other primates, however, the enzyme has been lost by some unknown mechanism. To identify the molecular basis for this loss, urate oxidase cDNA clones were isolated from pig, mouse, and baboon, and their DNA sequences were determined. The mouse urate oxidase open reading frame encodes a 303-amino acid polypeptide, while the pig and baboon urate oxidase cDNAs encode a 304-amino acid polypeptide due to a single codon deletion/insertion event. The authenticity of this single additional codon was confirmed by sequencing the mouse and pig genomic copies of the gene. The urate oxidase sequence contains a domain similar to the type 2 copper binding motif found in other copper binding proteins, suggesting that the copper ion in urate oxidase is coordinated as a type 2 structure. Based upon a comparison of the NH_2-terminal peptide and deduced sequences, we propose that the maturation of pig urate oxidase involves the posttranslational cleavage of a six-amino acid peptide. Two nonsense mutations were found in the human urate oxidase gene, which confirms, at the molecular level, that the urate oxidase gene in humans is nonfunctional. The sequence comparisons favor the hypothesis that the loss of urate oxidase in humans is due to a sudden mutational event rather than a progressive mutational process.**

Uric acid is catabolized to allantoin by urate oxidase, or uricase (E.C. 1.7.3.3.), in most vertebrates except humans, some primates, birds, and some species of reptiles (1). In mammals, this enzyme is localized predominantly in liver and is associated with the peroxisome as a tetramer with a subunit molecular mass of 32–33 kDa (2, 3).

Urate oxidase has attracted considerable interest for several reasons: (*i*) It has a unique evolutionary feature in that the enzyme has been lost during primate evolution with no obvious explanation (4, 5). (*ii*) The development of mice with complete hypoxanthine–guanine phosphoribosyltransferase deficiency that do not display any of the symptoms of Lesch–Nyhan syndrome has raised the possibility that the presence of urate oxidase in these mice may somehow protect them from neurological damage (6). (*iii*) The enzyme has been used as a peroxisomal marker (7) and is potentially a good system for studying protein sorting into peroxisomes (8).

The biological reason for the loss of urate oxidase activity in humans and certain primates is unknown. According to one view, this loss has had a distinctly beneficial effect. It has been shown that uric acid is a powerful antioxidant and a scavenger of free radicals; therefore, a high serum uric acid level caused by the loss of urate oxidase activity may have contributed to a decreased cancer rate and a lengthened hominoid life span (9). According to another view, however, the loss of urate oxidase is an unfortunate accident in the evolution of man and is primarily responsible for the metabolic disturbances leading to gouty arthritis and all its crippling and sometimes fatal complications (1).

We have previously described the cloning of a pig urate oxidase cDNA by a cDNA amplification procedure and have shown that although there is a homologous sequence in the human genome (10), no mRNA can be detected (11). These results have recently been confirmed by another group (12). The cDNA sequence of rat urate oxidase has recently been reported by three different groups (12–14); however, there are substantial differences in the published sequences. In this report we present the full-length sequence of pig urate oxidase cDNA and describe the isolation and characterization of two other urate oxidase cDNAs from mouse and baboon.¶ We have analyzed in detail the urate oxidase sequence differences between these species and clarified the differences in the rat sequences previously reported by others (12–14). A putative copper binding sequence is identified and the evolutionary implications are discussed. Finally, by showing that at least two premature chain termination mutations are found in the human urate oxidase gene, we confirm that the human urate oxidase gene is indeed inactive.

MATERIALS AND METHODS

Purification of Pig and Mouse Urate Oxidase, Preparation of Antiserum, and Immunoblotting. Partially purified pig liver urate oxidase was obtained from Sigma. Mouse liver urate oxidase was partially purified by the procedure of Conley and Priest (15) from mouse liver and then further purified to homogeneity by SDS/PAGE and electroelution according to the procedure of Hunkapiller *et al.* (16). Antiserum against pig liver urate oxidase was raised in goat (100 μg of the purified protein per injection) as described by Warr (17).

Immunoblotting was carried out by separating the proteins on an SDS/12% polyacrylamide gel and electroblotting to a nitrocellulose membrane (Bio-Rad) as described (18, 19). The membrane was incubated with the antiserum, and the antigen–antibody complexes were identified by ^{125}I-labeled protein A (NEN) according to Burnette (20).

Isolation of Urate Oxidase cDNA Clones and DNA Sequencing. Liver cDNA libraries of pig, mouse, and baboon were constructed in the λ ZAP vector (Stratagene) by using oligo(dT) priming by the procedure of Gubler and Hoffman (21). Isolation of an authentic pig urate oxidase cDNA probe (pUO-1) and the full-length pig urate oxidase cDNA have been previously described (10). pUO-1 was used to screen the mouse and baboon libraries at high stringency [5× SSC (1× SSC = 0.15 M sodium chloride/0.15 M sodium citrate, pH 7)/50% (vol/vol) formamide at 42°C] according to the procedure of Benton and Davis (22). DNA sequencing was performed by the Sanger dideoxy method (23).

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¶The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M27694–M27698).

Polymerase Chain Reaction. The genomic fragment at the codon deletion/insertion region of pig urate oxidase was isolated by the polymerase chain reaction according to Kogan *et al.* (24) by using 500 ng of genomic DNA as template. Thirty cycles of DNA polymerization (67°C, 3 min), denaturation (94°C, 30 sec), annealing (30°C, 1 min), and 5 units of *Thermus aquaticus* (*Taq*) DNA polymerase (Perkin–Elmer/Cetus) were used. Two oligonucleotide primers for polymerase chain reaction amplification were manufactured on an Applied Biosystems (ABI) 380B DNA synthesizer. The sequences of the primers are 5′-ACCCTCCCTGAGGTGAA-3′ (17-mer) and 5′-CCTTTGTCATAGGGCCCAGCAAATTTCT-3′ (28-mer).

Sequence Comparison. Comparison of the deduced amino acid sequences of pig, mouse, and baboon urate oxidase was based on the Poisson process as described elsewhere (25). Briefly, the proportion (p) of different amino acids between the two sequences and the expected number of amino acid substitutions per site can be related to each other by the Poisson process in probability theory. This simple mathematical model is accurate unless a very long evolutionary time span is considered. The formulations used in the calculation are $r = d/(2t)$, $p = n_d/n$, $d = -\log_e(1 - p)$, and $V(d) = p/[(1 - p)n]$, where r is the rate of amino acid substitution per year per site, d is the average number of amino acid substitutions per site, t is the evolutionary time, p is the proportion of different amino acids between the two sequences, $V(d)$ is the variance of d, n represents the total number of amino acids compared, and n_d is the number of different amino acids. In this comparison, deletions or insertions were ignored.

Isolation and Sequencing of the Human Urate Oxidase Gene. The human urate oxidase gene was isolated from a human genomic library (EMBL3) by using the pUO-b cDNA as a probe according to the method of Benton and Davis (22). DNA fragments containing individual exons were subcloned into pTZ18 or -19 (Pharmacia) and sequenced by the Sanger dideoxy method (23).

RESULTS

Analysis of cDNA Clones and Deduced Amino Acid Sequences. cDNA clones pUO-m (1.9 kilobases) and pUO-b (1.4 kilobases) were isolated from mouse and baboon liver cDNA libraries, respectively, by using pUO-1 as a probe. DNA sequencing of all three clones showed that each clone has a single open reading frame corresponding to 304 amino acids for pUO-p (pig) and pUO-b (baboon) and 303 amino acids for pUO-m (mouse). The molecular mass of the urate oxidase monomer, calculated from the deduced amino acid sequences, is 35,013 Da for pUO-p, 34,983 Da for pUO-b, and 35,044 Da for pUO-m. The predicted molecular masses are close to the observed molecular masses of the purified proteins (2, 3). Alignment of the cDNA sequences around the coding region and the deduced amino acid sequences are shown in Fig. 1. The sequences are highly conserved throughout the coding region; most of the amino acid changes are conserved substitutions. Several highly conserved domains are found (e.g., the amino acid sequence at positions 10–80 and 139–199).

A Putative Copper Binding Domain. Urate oxidase is known to be a copper binding protein (26, 27). Two different copper binding sequences have been well characterized in

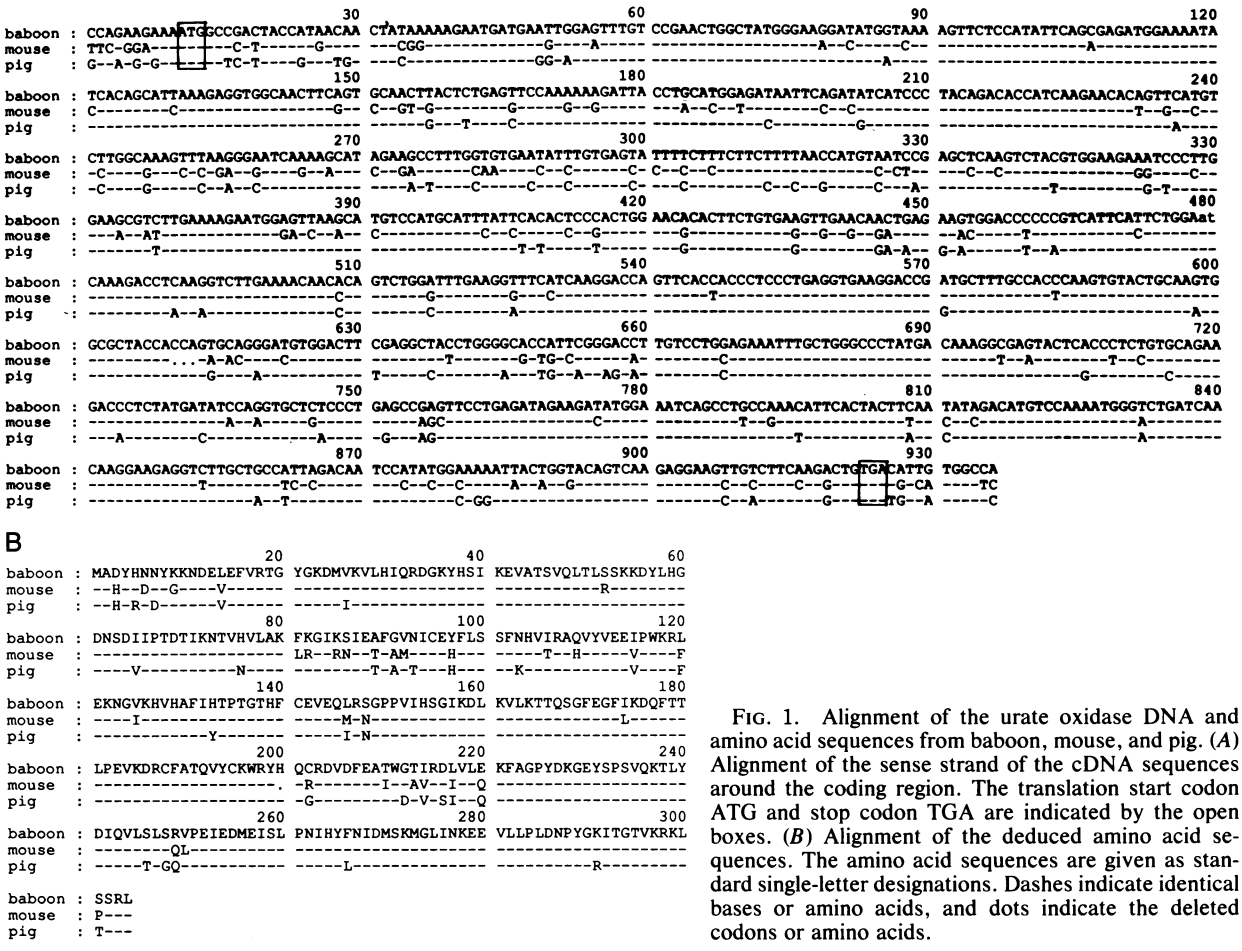


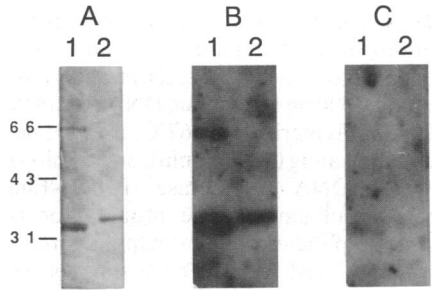
FIG. 1. Alignment of the urate oxidase DNA and amino acid sequences from baboon, mouse, and pig. (*A*) Alignment of the sense strand of the cDNA sequences around the coding region. The translation start codon ATG and stop codon TGA are indicated by the open boxes. (*B*) Alignment of the deduced amino acid sequences. The amino acid sequences are given as standard single-letter designations. Dashes indicate identical bases or amino acids, and dots indicate the deleted codons or amino acids.

UO	mouse	120	E	K	N	G	I	K	H	V	H	A	F	I	H	T	P	T	G	T	H	F	C	E	V	E
SOD	yeast	40	N	A	E	R	G	F	H	I	H	E	F	G	D	A	T	D	G	C	V	S	A	G	P	H
SOD	human	40	E	G	L	H	G	F	H	V	H	Q	F	G	N	D	T	A	G	C	T	S	A	G	P	H
SOD	bovine	40	E	G	D	H	G	F	H	V	H	Q	F	G	D	N	T	Q	G	C	T	S	A	G	P	H
Cp	human	87	L	H	T	V	H	F	H	G	H	S	F	Q	Y	K	H	R	G	V	Y	S	S	D	V	F
CCO	bovine	18	E	E	L	L	H	F	H	D	H	T	L	M	I	V	F	L	I	S	S	L	V	L	Y	I
CCO	human	18	E	E	L	I	T	F	H	D	H	A	L	M	I	I	F	L	I	C	F	L	V	L	Y	A

FIG. 2. Sequence similarity of the amino acid sequence of urate oxidase with the type 2 copper binding site as represented by bovine Cu/Zn superoxide dismutase and other copper binding proteins (29). The amino acid sequences are given as standard single-letter designations. The circled histidine residues are copper binding ligands that have been identified by x-ray crystallography (30, 31). Highly conserved amino acids are enclosed in the open boxes. The numbers at the start of each peptide identify the amino acid position within the complete protein. UO, urate oxidase; SOD, superoxide dismutase; Cp, ceruloplasin; CCO, cytochrome *c* oxidase.

other copper binding proteins and are known as type 1 and type 2 binding sites (28). The urate oxidase sequence at positions 121–144 shows a significant similarity to the type 2 binding site (Fig. 2). This type of copper binding site is represented by Cu/Zn-superoxide dismutase, in which the copper ion is bound to three nearby histidines and a fourth more distant histidine (30, 31). Urate oxidase shows the same double histidine binding motif as the Cu/Zn-superoxide dismutase (histidine-valine-histidine), while a histidine to glutamate change occurs at the position of the third histidine ligand. Several more histidine residues are found toward the COOH terminus in the sequence and could serve as the third and fourth possible copper binding ligands. The similarity between the type 2 binding sequence and sequences from other copper-containing proteins such as cytochrome *c* oxidase and ceruloplasin (29) suggests that the copper ions are bound in a similar fashion. In addition to the histidine residues, several other amino acids, such as glutamate, phenylalanine, and glycine are also conserved within the putative copper binding region.

A Codon Is Missing in Mouse Urate Oxidase Sequence. When compared to the baboon and pig urate oxidase sequences, a histidine residue is missing from the mouse urate oxidase sequence at amino acid position 200. This could result from either a deletion of a codon in the mouse or a insertion of a codon into the pig and baboon urate oxidase genes. The exact position of the deletion/insertion was estimated by the best sequence alignment. To exclude the possibility of a sequencing error and to further study the mechanism behind this event, urate oxidase genomic DNA clones were isolated from a mouse genomic library (X.W., unpublished data), and the corresponding exons were sequenced (Fig. 3). The pig urate oxidase genomic DNA corresponding to this region of the cDNA was also isolated by the polymerase chain reaction with two primers flanking the region (24). By sequencing the genomic clones, it was confirmed that it is indeed a deletion/insertion event since the exon sequences are identical to the corresponding cDNA



D Rat: Ala His Tyr His Asp Asp Tyr Gly
Pig: Asp Tyr Gly Lys Asn Asp Glu Val· · · · · ·

FIG. 4. The size and NH_2-terminal peptide sequence differences of urate oxidase. (*A–C*) Lane 1, purified pig urate oxidase. Lane 2, purified mouse urate oxidase. (*A*) Coomassie blue-stained SDS/polyacrylamide gel. (*B*) Immunoblot with antiserum against the pig urate oxidase. (*C*) Immunoblot with preimmune serum. Molecular size markers (in kDa) are indicated at right. (*D*) The NH_2-terminal amino acid sequences of the rat and pig urate oxidase (10, 13).

sequences. A trinucleotide repeat (ACCACC or CCACCA) is found in the pig urate oxidase sequence (both in the cDNA and the genomic clones) at the deletion/insertion region, whereas there is no such repeat in mouse urate oxidase sequence. This suggests that the trinucleotide repeat led to the deletion/insertion.

Posttranslational Modification of Pig Urate Oxidase. Although the three urate oxidase cDNA clones contain open reading frames of almost identical lengths, the pig urate oxidase is about 1 kDa smaller than that of the mouse when assessed by SDS/PAGE (Fig. 4*A*). The purified pig urate oxidase protein is reported to migrate at 32,000 Da (3), whereas the purified mouse urate oxidase protein exhibits a molecular mass of 33,000 Da, which is identical to the molecular mass of the rat urate oxidase (32). The identity of the proteins was confirmed by Western blotting with antiserum raised against pig urate oxidase (Fig. 4 *B* and *C*). A comparison of the NH_2-terminal peptide sequence between pig (10) and rat urate oxidase (13) demonstrates that the NH_2 terminus of pig urate oxidase is six amino acid residues shorter than that of the rat (Fig. 4*D*). We propose that posttranslational cleavage of pig urate oxidase leads to the size difference observed between pig and rodent (rat and mouse) urate oxidase.

Sequence Comparison and Implications in Evolution. The deduced amino acid sequences exhibit less than 10% diversity among the different species examined. Most substitutions are conserved changes; however, three substitutions found in the mouse sequence are quite unexpected (isoleucine to threonine at position 105, threonine to isoleucine at position 210, and serine or threonine to proline at position 301). These changes have been confirmed by sequencing the corresponding exons to exclude the possibility of cloning

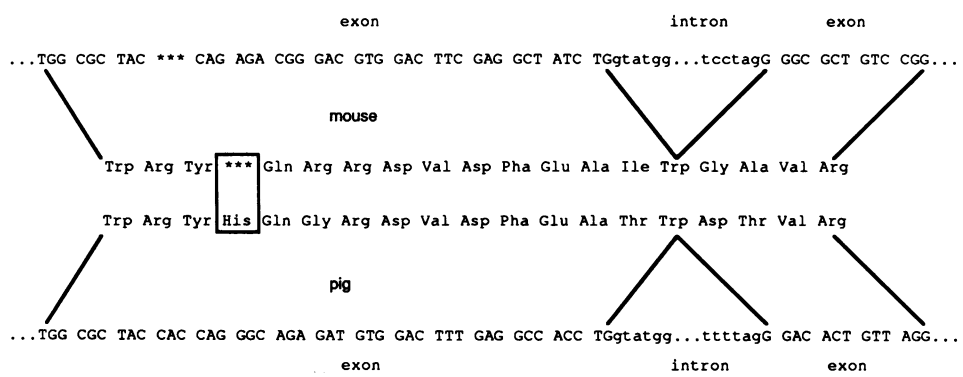


FIG. 3. The deletion/insertion site is within a single exon. Genomic DNA sequences and deduced amino acid sequences of mouse (*Upper*) and pig (*Lower*) urate oxidase at the codon deletion/insertion region. The exon sequence is in uppercase letters and the intron sequence is in lowercase letters. The asterisks indicate the deleted codon or amino acid. The site of the deletion/insertion has been determined by the best sequence alignment.

Table 1. Comparison of deduced urate oxidase amino acid sequences

	Baboon	Mouse	Pig
Baboon		30 (9.9%)	29 (9.5%)
Mouse	0.104 ± 0.019		32 (10.5%)
Pig	0.100 ± 0.019	0.111 ± 0.020	

The total number of amino acids compared is 304. The values in the upper right are the amino acid differences between two species (n_d). The numbers in parentheses are the percentages of the differences (p). The values in the lower left are the estimates of the average (± SD) number of amino acid substitutions per site between two species (d).

artifacts (X.W., unpublished data). Several highly conserved domains in the sequences suggest that these regions are important in the structure and function of the enzyme. The sequence comparison results based upon the Poisson process are summarized in Table 1 (25). Two conclusions can be drawn from these results. First, both amino acid differences (n_d) and the average amino acid substitutions per amino acid site (d) suggest that the urate oxidase sequence in baboon is still highly conserved, despite the fact that urate oxidase activity has been lost in humans and apes. Secondly, assuming that the species divergence between baboon and pig occurred approximately 60 million years ago (33) and that the mouse diverged from pig and baboon about 75 million years ago (34), the rate of amino acid substitutions of urate oxidase is estimated to be 0.7–0.8 × 10^{-12} amino acid substitutions per amino acid site per year. This value is below the average for a variety of proteins that have been examined to date (35). Thus, the selective pressure for urate oxidase has been maintained over the course of baboon evolution, and it appears unlikely that the accumulation of multiple mutations accounts for the loss of urate oxidase activity in humans.

Two Nonsense Mutations in the Human Urate Oxidase Gene. Three λ clones of the human urate oxidase gene were isolated, and the sequences of four exons were determined. Two nonsense mutations were identified at amino acid positions 33 and 187. In both cases, codons for arginine (CGA or AGA) gave rise to stop codons (TGA) (Fig. 5). The presence of these premature stop codons in the coding region clearly indicates that the urate oxidase gene in human is inactive.

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                    11
ctgtgttttcattgcag AAT GAT GAG GTG GAG TTT GTC CGA ACT GGC TAT GGG AAG
                    asn asp glu val glu phe val arg thr gly tyr gly lys
                                  31
GAA ATG GTA AAA GTT CTC CAT ATT CAG TGA GAT GGA AAA TAT CAC AGC ATT
glu met val lys val leu his ile gln stop asp gly lys tyr his ser ile
                                      51
AAA GAG GTG GCA ACT TCA GTG CAA CTT ACT CTA AGT TCC AAA AAA GAT TAC
lys glu val ala thr ser val gln leu thr leu ser ser lys lys asp tyr
                                                  71
CTG CAT GGA GAT AAT TCA GAC ATC ATC CCT ACA GAC ACC ATC AAG AAC ACA
leu his gly asp asn ser asp ile ile pro thr asp thr ile lys asn thr

GTT CAT GTC TTG GCA AAG TTT AAA GAA gtatgtgtcacttcttc
val his val leu ala lys phe lys glu

                149
tacttgaaacag GGA CCC CAA GTC ATT CAT TCT GGA ATC AAA GAC CTC AAG GTC
              gly pro gln val ile his ser gly ile lys asp leu lys val
                                169
TTG AAA ACA ACA CAG TCT GGA TTT GAA GGT TTC ATC AAG GAC CAG TTC ACT
leu lys thr thr gln ser gly phe glu gly phe ile lys asp gln phe thr
                                          189
ACC CTC CCT GAG GTG AAG GAC TGA TGC TTT GCC ACC CAA GTG TAC TGC AAG
thr leu pro glu val lys asp stop cys phe ala thr gln val tyr cys lys
                                                  209
TGG CGC TAC CAC CAG TGC AGG GAT GTG GAC TTC AAG GCT ACC TG gtatgaac
trp arg tyr his gln cys arg asp val asp phe lys ala thr trp
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FIG. 5. The sequences of two exons from the human urate oxidase gene contain the two nonsense mutations. The exon sequence is in uppercase letters and the intron sequence is in lowercase letters. The deduced amino acid sequence is shown below the DNA sequence. The nonsense mutations are in boldface letters. The numbers above the sequence are the amino acid positions corresponding to the baboon urate oxidase.

DISCUSSION

We have isolated and sequenced three urate oxidase cDNA clones from three mammalian species: pig, baboon, and mouse. The corresponding sequence in clone pUO-p matches the sequence of pUO-1, which we had previously determined (10). The sequences we present here contain the same open reading frame and approximately 90% sequence identity with the rat sequence recently reported by Reddy *et al.* (12). This suggests that the substantial sequence differences between various published rat urate oxidase sequences is likely due to sequencing errors that result in frame shifts and shortened open reading frames (13, 14).

A histidine residue is missing not only in the mouse urate oxidase but also in the rat urate oxidase sequence. This deletion/insertion site is found within a single exon and thus the possibility of it being due to an aberrant splicing event is unlikely. The trinucleotide repeat within this region suggests that the loss or gain of a codon could have resulted from a duplication or deletion of one of the trinucleotides at the level of DNA replication or recombination. Similar trinucleotide repeats have been shown to lead to deletions in some other systems, such as the mutation of human hypoxanthine–guanine phosphoribosyltransferase gene in a Lesch–Nyhan patient (36) and the mutation in hamster adenosine phosphoribosyltransferase locus (37).

The phylogenetic relationship of the mouse, pig, and baboon species is controversial (38, 39). Since both the pig and baboon urate oxidase contain this histidine residue, this would suggest that baboons are in fact evolutionally closer to pigs than to mice. This is supported by the fact that the human gene also contains this residue. A phylogenetic tree based upon the progressive alignments of the urate oxidase sequences of mouse, pig, and baboon would also support this conclusion (X.W., unpublished data).

In vitro studies of rat liver urate oxidase have shown that the enzyme is synthesized on free polysomes and translocated into peroxisomes without posttranslational processing (8). Based upon indirect evidence, we suggest that the maturation of pig liver urate oxidase involves the posttranslational cleavage of a six-amino acid leader peptide from the NH_2 terminus. The sequence being cleaved is located within a less conserved region. Although the function of this cleavage is unclear, it does not seem to reduce the enzyme activity (26). One possible explanation is that a proteolytic cleavage site was generated in the pig urate oxidase sequence due to random mutations that have no biological effect.

Urate oxidase is a copper-containing enzyme (26, 27); however, the structure of the copper binding region is unknown. We have shown in this report that the urate oxidase sequence contains a region that is very similar to a type 2 copper binding site, suggesting that the urate oxidase sequence at amino acid positions 121–144 is the copper binding site and that the copper ion in urate oxidase is coordinated as a type 2 structure. The sequence similarity of several copper proteins with the type 2 binding site (29) indicates that this sequence may have an ancient evolutionary origin. It has been argued that functional domains of proteins are encoded by individual exons and that one mechanism of protein evolution is the sorting and shuffling of exons (40). However, the putative copper binding sequence in the mouse urate oxidase is encoded in two exons separated after the lysine and before the glycine residue at amino acid position 124 (X.W., unpublished data). This suggests that the conserved glutamate residue may not be important for the copper binding function.

It has been shown that Old World monkeys and some New World monkeys have appreciable urate oxidase activity, but the specific activities are only 50% or less of that of the mouse (4, 5); however, the reason for this decrease is unknown. We

reasoned that the decrease in urate oxidase activities could be due to either an increase in the mutation rate in the protein itself or to mutations leading to a decrease in the transcription rate or the level of transcripts of the urate oxidase gene during primate evolution. Our sequence data indicate that the mutation rate has not increased in the baboon, suggesting that the selective pressure to maintain urate oxidase activity during baboon (an Old World monkey) evolution has been quite high. We have not examined the transcription rate of the urate oxidase gene in the baboon or other species; however, our preliminary results indicate that the decrease (if any) in the steady-state level of urate oxidase mRNA in the baboon is insignificant (X.W., unpublished data). A total of 13 amino acids are unique to the baboon urate oxidase sequence; however, most of these are conserved substitutions. Thus, the decrease in the enzyme activity is presumably due to minor amino acid changes. These mutations could have occurred in the common ancestor of the New World and the Old World monkeys, which would result in the observed decreases in urate oxidase specific activity seen in the whole primate family.

The loss of urate oxidase in humans is an evolutionally recent event, occurring during the evolution of primates; however, the basis of this loss remains controversial. Two mechanisms have been proposed based upon protein studies. The first possibility is that a series of stepwise mutations during primate evolution led to a progressive loss of the urate oxidase activity (4). The alternative possibility is that a single mutation event silenced the urate oxidase gene in a common ancestor to the five living genera of hominoids after the divergence from Old World monkeys (5). To investigate these two possibilities, we have addressed the question at a molecular level. We compared the sequence of baboon, pig, and mouse urate oxidase cDNAs. The progressive mutation hypothesis is supported by the fact that, while some New World monkeys are found to have various amounts of urate oxidase activity, others have no detectable urate oxidase activity (4, 5, 32). Conversely, in support of the single mutation event hypothesis, the baboon urate oxidase sequence does not show any evidence of an increase in the mutation rate. Furthermore, we have shown that the loss of urate oxidase activity in humans may be due to the loss of gene transcription or processing (10, 11). The single mutation hypothesis is also supported by the fact that the urate oxidase sequence of humans is highly homologous to the baboon sequence (96%; X.W., unpublished data). This high degree of homology further suggests that the loss of urate oxidase in humans occurred more recently than had been previously thought and is likely due to a sudden mutation event that inactivated the gene at the level of transcription or processing.

The correct identification of the original mutation that inactivated the human gene is complicated by the accumulation of secondary mutations in the inactive gene. Since the simultaneous occurrence of two nonsense mutations is unlikely, we presume that the two nonsense mutations we have found were acquired sequentially.

The relationship between the loss of urate oxidase activity in humans and some New World monkeys is not clear. Since New World monkeys diverged earlier from the hominoids than Old World monkeys (41), it is unlikely that urate oxidase activity would have been lost in humans and some New World monkeys due to the same mutational event, whereas in Old World monkeys its enzymatic activity and sequence is still conserved. Although the complete sequence of the human urate oxidase gene and that of other primates is not known, it is possible that the loss of urate oxidase in humans and some New World monkeys is the result of independent mutation events.

We are grateful to Dr. William Craigen for help in preparing the manuscript; to Andrew Civitello for technical assistance; to Drs. David L. Nelson, Jeffrey Chamberlain, Grant MacGregor, and Richard Gibbs for helpful suggestions; to Dr. William O'Brien for providing baboon RNA; and to Dr. John Weis for the human genomic library. This work was supported by Public Health Service Grant DK31428, Welch Foundation Grant Q-533, and the Howard Hughes Medical Institute.

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